Therapeutic drug monitoring: how to improve moxifloxacin exposure in tuberculosis patients
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 8

Summary
Tuberculosis (TB) is the world’s deadliest infectious disease with a larger annual death toll today than even HIV/AIDS. Global migration, changing comorbidity profiles (e.g. diabetes), HIV/AIDS, but in particular the spread of multidrug-resistance (MDR) is challenging the ending of the TB pandemic. Isoniazid (INH) and pyrazinamide (PZA) are first-line drugs, but also well-known hepatotoxic, and because of drug resistance and drug intolerance, clinicians are increasingly forced to use second-line drugs. Efficacy against *M. tuberculosis* (MTB) and tolerability of these 2nd line agents has been less studied than of 1st line drugs. With a treatment success rate of 54% (reported under research conditions) or less (under real life conditions) and reported drug-susceptibility rendering TB untreatable, there is a dire need to improve MDR/rifampicin-resistant (RR) TB treatment.

The global response to these problems has been to develop shorter treatment regimens for drug-susceptible and MDR/RR-TB, in order to improve drug-adherence, or by introducing new 2nd line drugs. Bedaquiline was the second drug ever approved for treatment of TB (2012), limited for MDR-TB, following rifampicin (RIF) in 1968. However, variability of exposure to the fixed-dosed anti-TB drugs is highly likely to happen in real life, as a result of potential drug-drug interactions (DDIs), and considering the impact of malnutrition and other comorbid disease conditions (HIV, diabetes). We therefore proposed that more knowledge on the clinical pharmacology of anti-TB drugs might help to improve treatment success and to preserve the limited number of powerful drugs for treating TB.

Moxifloxacin (MFX) was the focus of this thesis. This later-generation fluoroquinolone (FQ) was introduced approximately a decade ago in our TB centre (Beatrixoord, UMCG), which serves as one of the two referral centres in the Netherlands, based on promising *in vitro* and *in vivo* data. MFX is currently a key component of MDR/RR-TB treatment and in case of intolerance against first-line anti-TB drugs. In general, the standard MFX dose of 400mg QD is recommended for TB. MFX is a comparator drug in pharmacological studies addressing QTc prolongation, which is a potential concern for wider use.

In this thesis, we aimed to contribute to the knowledge of pharmacokinetics (PK) and pharmacodynamics (PD) of MFX in TB in order to improve treatment success. Our second aim was to develop therapeutic drug monitoring (TDM) diagnostics to give health care professionals the ability to measure MFX concentrations and to adjust the dose to optimally treat TB in individual patients.
The role of fluoroquinolones for tuberculosis

In Chapter 2A, we performed a literature search on the PK, PD and DDIs of 14 FQs for drug-resistant TB, including 9 FQs not yet used for TB, e.g. clinafloxacin, garenoxacin, lomefloxacin, sitafloxacin, sparfl oxacin, trovafloxacin, gemifloxacin, grepafloxacin and DC-159a. At the time of the literature study (2011), levofloxacin (LFX) was the preferred FQ for TB, gatifloxacin (GFX) was only recommended as last remedy, and the older generation FQs ciprofloxacin (CFX) and low-cost ofloxacin (OFX) were becoming less popular due to low efficacy. MFX was not recommended, because of limited data on long-term safety.

We evaluated the 14 FQs for bioavailability, drug-food interactions, protein binding, distribution into the alveolar macrophages (AM) and epithelial lining fluid (ELF), dosing interval, factors in which dose adjustment is needed (i.e. renal- and hepatic function, age, gender), in vitro and in vivo susceptibility against drug-resistant MTB strains, and DDIs. In accordance with the core position of FQs, i.e. MFX, GFX and LFX, in the current WHO guidelines for drug-resistant TB (2016), we revealed that particularly MFX should be a far more important drug in the treatment of a drug-resistant TB patient, or in case of intolerability against first-line drugs, based on an estimated high bactericidal and sterilizing efficacy, a high concentration in the AM, ELF, cerebrospinal fluid (CSF) and bone tissue, relative to plasma, and benefits such as no dose adjustment in patients with an impaired renal and hepatic function and a once-daily dosing interval. We also noted that 600 or 800mg QD instead of 400mg QD is probably needed to prefer MFX above other FQs. RIF and also drugs that prolong the QT interval are a threat for successful use of MFX, resulting in approx. 30% reduction of MFX exposure or an extra risk for Torsade the Points, respectively. TDM and/or ECG monitoring might thus be needed to safeguard its use. Of the novel FQs, DC-159a, a structure analogue of MFX, was defined as most promising FQ, but also sparfl oxacin (SFX), sitafloxacin (STX) and trovafloxacin (TFX) were defined as high-potential drugs for TB based on PK/PD.

In the following years, particularly, simplified, shorter regimens for pulmonary TB and intensified regimens for TB meningitis (TBM) became a major topic of TB research regarding MFX, GFX and/or LFX. This direction suits their PK/PD properties (Chapter 2A). The most powerful anti-TB drugs RIF, MFX and GFX have the potential to kill the persistent subpopulation of MTB in the hypoxic pulmonary lesions, and thus the potential to induce a shorter TB treatment. Also, the favourable penetration into CSF suggests a role for LFX and MFX in TB meningitis, i.e. intensification of the first weeks of treatment with these FQs could improve overall survival. In 2018, we summarized the treatment outcomes of the clinical trials
investigating the role of MFX, GFX and LFX for TB considering the PK/PD considerations mentioned earlier.

In Chapter 2B no new publications on the four selected high-potential FQs for TB (i.e. DC-159a, SFX, STX, TFX) were retrieved in the literature. Shortened TB regimens including MFX or GFX were not favourable. Of the pulmonary DS-TB patients, 75-90% was successfully treated with a 4-month regimen including MFX or GFX, but this was inferior to standard 6-month treatment. We criticized the predictions on the pre-clinical study designs used, e.g. the absence of lesion heterogeneity in BALB/c mice, and the ignorance of a potential sub-optimal drug-exposure at the side of infection based on the already available PK/PD information for MFX, e.g. PK/PD variability, the PK DDI and paradoxical PD DDI between RIF and MFX, and the already suggested higher dose of 800mg QD needed for successful kill of mycobacteria in log-phase growth. Our discussion on the evidence of the short-course MDR/RR-TB regimen based on GFX or MFX – still 9 to 12 months – also focussed on optimal drug-exposure as in the Bangladesh cohort some patients were treated with high-dose GFX. For TBM there is no evidence-based treatment regimen available, but the LFX and MFX concentrations found in CSF are encouraging. In one of the three clinical trials, treatment success was in favour of the FQ (LFX).

The overall conclusion of Chapter 2B was that the promising PK/PD properties of MFX, GFX and LFX should be exploited to attain the goal of optimized dosing.

**Moxifloxacin**

Several years before the start of the studies described in this thesis, MFX was introduced in our TB centre (Beatrixoord, UMCG) based on promising *in vivo* and *in vitro* bactericidal effects against MTB.

To gain knowledge on MFX PK in TB patients we developed and validated a liquid chromatography tandem-mass spectrometry (LC-MS/MS) method in plasma and CSF according to the Food and Drug Administration (FDA) guidelines for bio-analytical method validation (Chapter 3). The pre-treatment procedure of this method was simple and fast. We conducted a cross-validation for the protein-unbound fraction of MFX in plasma based on the method in CSF as a change in protein binding might influence MFX efficacy. The following mass transitions were measured to qualify MFX *m/z* 402.0 to *m/z* 358.2 and the internal standard (cyanoimipramine) *m/z* 306.0 to *m/z* 218.0. INH, RIF, ethambutol and linezolid were not interfering with the peaks of MFX and the internal standard. Calibration curves in plasma
and CSF were linear over the range of 0.05 – 5 mg MFX/L (R² > 0.99). Bias and coefficients of variation (CV, between-run and within-run) were < 20% for the Lower Limit Of Quantification (LLOQ) and < 15% for the low, medium and high Quality Control (QC) samples in plasma and CSF as required by the FDA. The same was true for the low, medium and high QC samples in plasma ultrafiltrate. The CVs of the recovery of MFX in plasma and CSF applied with the FDA criteria, but the results were remarkably high, i.e. 114.2-124.8%.

The anticoagulant EDTA, present in the blood collection tube, was identified as a potential cause. Clinical application of the method to patient care was successfully tested with a patient curve in plasma and CSF. The protein-unbound fraction of MFX was also identified.

The promising bactericidal activity of MFX encouraged us to retrospectively review the medical charts of the TB patients already treated with MFX in Beatrixoord. In Chapter 4A, we therefore retrospectively evaluated efficacy, safety/tolerability and DDIs of MFX, in relation to PK, as part of TB treatment. Between January 2006 and January 2009 89 patients were treated with 400mg MFX QD (median, 6.9 mg/kg) at Beatrixoord for a median (IQR) period of 74 (29-186) days. Ten patients had a known HIV co-infection and 10 patients were diagnosed with diabetes mellitus.

In 48 patients, MFX was prescribed temporarily because of a MDR-TB risk based on treatment history, while waiting for a resistance pattern, or because of transient intolerability of first-line anti-TB drugs. Hypersensitivity (n=1) or gastrointestinal complaints (n=2) were reasons to stop MFX based on adverse events. A routine 3-lead ECG was obtained after ~2 weeks of 400mg MFX QD or in case of dose-escalation. QT interval prolongation was not observed. As PK sampling was not performed in every patient, we were not able to relate the area under the concentration-time curve (AUC₀⁻₂₄h) to the limited adverse events.

A full plasma concentration-time curve was available for sixteen patients. Our retrospective study pointed out a unique 9-fold variability in AUC₀⁻₂₄h on 400mg QD in TB patients. Also, the plasma protein binding (n=9) was highly variable, which is important as only the unbound fraction has an antimicrobial effect. No significant difference (p=0.104) of MFX AUC₀⁻₂₄h was observed between patients with or without RIF concomitant treatment of MFX.

Our retrospective study showed that TB treatment regimens containing MFX were well tolerated for a prolonged period of time. Furthermore, we concluded that based on a 9-fold PK variability, 400mg MFX QD, used off-label, is probably not sufficient for every TB patient. Given a target AUC₀⁻₂₄h/MIC of 100 and a median AUC₀⁻₂₄h of 24.8 mg*h/L, a dose of 400mg QD is too low for patients infected with an isolate with a MIC ≥ 0.25 mg/L. In 4 patients, the dose was actually increased from 400mg QD to 800mg QD based on a low AUC₀⁻₂₄h and/or (expected) high MIC value. Since the median AUC₀⁻₂₄h in our TB patients was low compared
to healthy volunteers, a dose-escalation tended to be safe along with ECG monitoring around the expected peak-level.

In Chapter 4B, we hypothesized that TB disease activity and the clinical condition of a TB patient, including comorbid conditions (HIV, diabetes), influences MFX PK. Time elapsed since start of TB treatment was defined as surrogate parameter of stage of TB disease. We retrospectively evaluated MFX PK in the treatment period January 2006 – January 2013, and studied known determinants of MFX AUC$_{0-24h}$ (i.e. RIF DDI); putative determinants related to disease severity and/or clinical condition (i.e. BMI<18.5, time elapsed since start of TB treatment, Erythrocyte Sedimentation Rate (ESR), C-reactive protein, diabetes mellitus, HIV co-infection); and gender and geographical region of origin as possible confounders.

Of the 211 TB patients treated with 400mg MFX QD, a MFX AUC$_{0-24h}$ was available in 39 patients (23 MDR-TB patients). The AUC$_{0-24h}$ ranged from 10 to 73 mg*h/L in this cohort. Using multivariable linear regression analysis, a RIF DDI (p=0.004) and gender (p=0.019) remained significantly associated with MFX AUC$_{0-24h}$ after adjustment for potential confounders, whereas a HIV co-infection (p=0.091) and time passed since start of TB treatment lost significance. However, a disproportional increase of MFX AUC$_{0-24h}$ was observed in some patients with multiple MFX AUC$_{0-24h}$ values over time. We therefore concluded that we might have unjustly assumed that every patient in Beatrixoord has an insufficiently high to improve overall survival. In a letter to the editor, we therefore encouraged the authors to do an extended PK/PD analysis of their data set, in order to relate

Chapter 4B also pointed out a relatively low MFX AUC$_{0-24h}$ and peak-level for males compared to females, which is unique to this study. This result suggests a benefit of concentration monitoring for male patients. A prospective crossover study of oral and intravenous administration of MFX is needed to test our hypothesis that there is a gender-difference in absolute bioavailability, possibly due to a difference in disease related intestinal dysfunction.

Earlier we mentioned the promising role of MFX for TBM. However, in the last five years, only one clinical trial investigated high-dose MFX therapies at the early phase of TBM treatment (Chapter 2B). The investigators found no survival benefit of MFX for TBM. However, this clinical trial was designed to explore PK and safety of high-dose MFX and/or high-dose RIF therapies. Drug exposure –though increased– might still have been insufficiently high to improve overall survival. In a letter to the editor, we therefore encouraged the authors to do an extended PK/PD analysis of their data set, in order to relate
cumulative drug exposure of MFX and RIF (and INH) with overall survival, using a receiver operating characteristic (ROC) analysis (Chapter 4C).

**Therapeutic drug monitoring**

TDM may be an appropriate tool for dose-optimization. Therefore, a good prediction of the plasma-concentration profile is mandatory, but often requires multiple venous blood samples. This procedure is time-consuming, expensive and a burden to the patient. To overcome these problems, in Chapter 5 we focussed on a limited sampling strategy (LSS) based on population PK.

The MFX (400mg QD) population PK parameters were generated based on rich PK profiles of 21 TB patients. Given the small sample size, we chose an iterative two-stage Bayesian procedure, starting with literature-based estimates. The CVs of the LC-MS/MS method (Chapter 3) and potential model misspecification (e.g. biological variation in plasma-clearance) were taken into account. The AUC$_{0-24h}$ (400mg QD) values of the patient omitted during leave-one-out (n-1) validation were overestimated with 0.6% (IQR, -2.8% – 5.9%). RIF was not a confounder of MFX population PK.

A Monte Carlo simulation of 1,000 random patients drawn from our cross-validated population PK model was used to select the best LSSs. The best LSS for clinical practice, based on a sample 4 and 14 hours post MFX dosage, led to an accurate (bias < 5%) and precise (Root Mean Square Error < 15%) prediction of the plasma-concentration profile of MFX for TB patients. We also pointed out that lag-time was a confounder of adequate prediction of population PK and therefore it is not surprising that the peak-level was not included in our best LSSs.

In TB endemic areas devoid of advanced PK technology, a low-tech TDM tool may be useful for timely detection of a too low drug-exposure, especially for cornerstone anti-TB drugs. In Chapter 6, we therefore used the aromatic core of FQs to detect MFX exposure in oral fluid based on fluorescent intensity at a wavelength of 366 nm, using a simple semi-quantitative Thin Layer Chromatography (TLC) method. INH, RIF, pyrazinamide, ethambutol, kanamycin, clarithromycin and linezolid revealed no fluorescent emission.

An inter-observer error in visual read-out was expected to be the biggest potential flaw, but there were no published statistical methods to sustain a correct and precise result, using our TLC method. We adequately validated the robustness of our method using a latent variable approach. A moderate to strong agreement in visual read-out was suggested based on an
The intraclass correlation coefficient (ICC) was 0.968 (95% CI, 0.714-0.997). Given an average weighted kappa of at least 0.84, classification of a patient sample was expected to be correct in most cases, using our method. In Chapter 6, we also discussed the two different ways of judging the intensity of a patient sample, i.e., starting read-out with the reference category with the highest or lowest intensity, but the robustness was not expected to be affected.

Seven paired concentration-time curves in plasma and oral fluid were also available and used to test the clinical applicability of our method. A peak-level in oral fluid categorized > 0.5 mg MFX/L was suggested to be related to a plasma AUC\(_{0-24h}\) of at least 20 mg*h/L.

We concluded that our simple and non-invasive method may improve MFX exposure, but it has to proof its benefit in local TB programs in resource-limited areas.

**General discussion and future perspectives**

In the general discussion (Chapter 7), we discussed three parts of this thesis: the right pre-clinical study design to test bactericidal and sterilizing efficacy of a FQ for TB, the use of a fixed MFX dose for all TB patients, and the practical issues of TDM. We argued that increased knowledge on PK/PD targets and further steps to identify patients at risk for a too low (or too high) drug-exposure may help to improve MFX exposure for TB. We also pointed out the proposed important role of the recently (2017) setup WHO task force on the PK/PD of anti-TB drugs and the Foundation for Innovative New Diagnostics (FIND), or other international organisations, in personalized dosing.

We concluded that our thesis contributed to the knowledge on the clinical pharmacology of MFX for TB and our results show that with TDM TB treatment can be improved.
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